LIMIT TESTS

Limit test is defined as quantitative or semi quantitative test designed to identify and control small quantities of impurity which is likely to be present in the substance.

Limit test is generally carried out to determine the inorganic impurities present in compound.

In short, limit test is nothing but to identify the impurities present in the substance and compare it with standard.

Limit test for chlorides, sulphates, iron, lead and heavy metals are carried out in Nessler cylinders which are made of colourless and have uniform diameter and height.

Two cylinders are required each time *ie* one for the "Test" (sample) and other for the "Standard".

Standards are kept constant but the quantities of sample are varied according to the limits of impurities prescribed in pharmacopoeia.

Limit test is performed in distilled water or purified water bcz ordinary tap water contains number of ions to vitiate the test

LIMIT TEST OF CHLORIDE

Principle:

Limit test of chloride is based on the interaction of chlorides with silver nitrate in presence of dilute nitric acid to form silver chloride.

When only very small quantity of chloride ions are present, silver chloride appears as Opalescence (semi translucent white) in the solution and not as precipitate.

$$NaCl + AgNO_3 \xrightarrow{HNO_3} AgCl + NaNO_3$$

Procedure:

Test sample	Standard compound
Specific weight of compound is dissolved in 10 ml of water or solution is prepared as directed in the pharmacopoeia and transferred in Nessler cylinder labelled as "Test"	Take 1ml of 0.05845 % W/V solution of sodium chloride in Nessler cylinder labelled as "Standard". add 10ml of water
Add 10ml of Nitric acid	Add 10 ml of Nitric acid
Dilute to 50ml in Nessler cylinder	Dilute to 50ml in Nessler cylinder
Add 1ml of $AgNO_3$ solution	Add 1ml of $AgNO_3$ solution

Stir each solution with a glass rod and allow to stand for 5 minutes. Compare the opalescence transversly against a dark background to have a better contrast for comparison.

Observation:

If opalescence produces in Test (sample solution) is less than the standard solution, the given sample passes the limit test of chloride and also the given sample complies with IP

The opalescence produce in "Test" (sample) is more as compared to the "Standard", means sample contain more quantity of chloride(impurity) than prescribed limit. , the given sample fails the limit test of chloride and also the given sample does not complies with IP

- Nitric acid is added in the limit test of chloride to prevent the precipitation of other acid with $AgNO_3$ bcz in presence of HNO_3, other precipitates are not produced and only chlorides get precipitated.

LIMIT TEST OF SULPHATE

Principle:

Limit test of sulphate is based on the interaction of sulphate with barium chloride in presence of dilute hydrochloric acid to form barium sulphate which appears as solid particles (turbidity) in the solution.

Procedure:

Test sample	**Standard compound**
Specific weight of compound is dissolved in 10ml of water or solution is prepared as directed in the pharmacopoeia and transferred in Nessler cylinder labelled as "Test"	Take 1ml of 0.1089 % W/V solution of potassium sulphate in Nessler cylinder labelled as "Standard",add 10 ml of water
Add 2ml of dilute hydrochloric acid	Add 2ml of dilute hydrochloric acid
Dilute to 45 ml in Nessler cylinder	Dilute to 45 ml in Nessler cylinder
Add 5ml of barium sulphate reagent	Add 5ml of barium sulphate reagent
Stir with glass rod and Keep aside for 5 min	Stir with glass rod and Keep aside for 5 min
Observe the Turbidity	Observe the Turbidity

According to 1985 IP, instead of barium choride solution ,barium sulphate reagent is used for limit test for sulphate

- Barium sulphate reagent contains barium chloride, sulphate free alcohol and small amount of potassium sulphate.
- Potassium sulphate is used to increase the sensitivity of the test by giving ionic concentration in the reagent.

- Alcohol helps to prevent super saturation Hydrochloric acid helps to make solution acidic.

Observation:

If turbidity produces in Test (sample solution) is less than the standard solution, the given sample passes the limit test of sulphate and also the given sample complies with IP

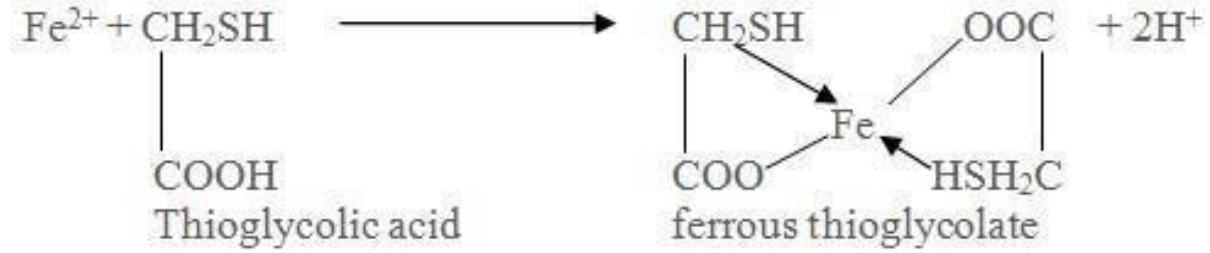
The turbidity produce in "Test" (sample) is more as compared to the "Standard", means sample contain more quantity of sulphate (impurity) than prescribed limit. , the given sample fails the limit test of sulphate and also the given sample does not complies with IP

- HCl is added in the limit test of sulphate to prevent the precipitation of other acid with $BaCl_2$.bcz in presence of HCl, other precipitates are not produced and only sulphate get precipitated

LIMIT TEST OF IRON

Principle:

Limit test of Iron is based on the reaction of iron in ammonical solution with thioglycollic acid in presence of citric acid to form ferrous thioglycolate which is pale pink to deep reddish purple in color.



Procedure:

Test sample	Standard compound
Sample is dissolved in specific amount of water and then volume is made up to 40 ml	2 ml of standard solution of iron diluted with water upto 40ml
Add 2 ml of 20 % w/v of citric acid (iron free)	Add 2 ml of 20 % w/v of citric acid (iron free)
Add 2 drops of thioglycollic acid	Add 2 drops of thioglycollic acid
Add ammonia to make the solution alkaline and adjust the volume to 50 ml	Add ammonia to make the solution alkaline and adjust the volume to 50 ml
Stir with glass rod and Keep aside for 5 min	Stir with glass rod and Keep aside for 5 min

Compare the colour in the two Nessler's cylinder by viewing vertically downwards. Earlier amonium thiocyanate reagent was used for the limit test of iron. Since thioglycolic acid is more sensitive reagent, it has replaced ammonium thiocyanatein the test.

Observation:

The intensity of purple color produce in TEST(sample solution)is less than STD(standard solution), the sample will pass the limit test of iron and vice versa.

Reasons:

- Citric acid prevents precipitation of iron with ammonia .it keeps iron in solution form even in presence of ammonia by forming a complex with it.
- Ammonia to make solution alkaline

<u>Functions of Thioglycolic acid</u>

- Thioglycolic acid helps to convert trivalent ferric form to divalent ferrous form
- Thioglycolic acid produces purple colour with ferrous ion in ammonical alkaline medium

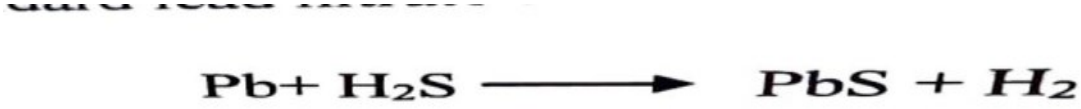
LIMIT TEST OF HEAVY METAL

Principle:

Limit test of heavy metals is based on the reaction of metallic impurities with hydrogen sulfide in acidic medium to form brownish colour solution.

Metals that response to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.

The metallic impurities in substances are expressed as parts of lead per million parts of the substance. The usual limit as per Indian Pharmacopoeia is 20 ppm



Procedure:

The Indian Pharmacopoeia has adopted three methods for the limit test of heavy metals.

Method I: Use for the substance which gives **clear colorless** solution under the specific condition.

Test sample	**Standard compound**
Solution is prepared as per the monograph and 25 ml is transferred in Nessler‟s cylinder	Take 2 ml of standard lead solution and dilute to 25 ml with water
Adjust the pH between 3 to 4 by adding dilute acetic acid or dilute ammonia solution	Adjust the pH between 3 to 4 by adding dilute acetic acid or dilute ammonia solution
Dilute with water to 35 ml	Dilute with water to 35 ml

Add freshly prepared 10 ml of hydrogen sulphide solution	Add freshly prepared 10 ml of hydrogen sulphide solution
Dilute with water to 50 ml	Dilute with water to 50 ml
Allow to stand for five minutes	Allow to stand for five minutes
View downwards over a white surface	View downwards over a white surface

Observation:

The color produce in TEST(sample solution) should not be greater than STD (standard solution). If color produces in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa.

Method II: Use for the substance which **do not give clear colorless** solution under the specific condition.

Principle:

Limit test of heavy metals is based on the reaction of metallic impurities with hydrogen sulfide in acidic medium to form brownish colour solution

Test sample	**Standard compound**
Weigh specific quantity of test substance, moisten with sulphuric acid and ignite on a low flame till completely charred Add few drops of nitric acid and heat to 500 °C Allow to cool and add 4 ml of hydrochloric acid and evaporate to dryness Moisten the residue with 10 ml of hydrochloric acid and digest for two minutes Neutralize with ammonia solution and make just acid with acetic acid	Take 2 ml of standard lead solution and dilute to 25 ml with water
Adjust the pH between 3 to 4 and filter if necessary	Adjust the pH between 3 to 4 by adding dilute acetic acid „Sp“ or dilute ammonia solution „Sp“
Dilute with water to 35 ml	Dilute with water to 35 ml
Add freshly prepared 10 ml of hydrogen	Add freshly prepared 10 ml of

sulphide solution	hydrogen sulphide solution
Dilute with water to 50 ml	Dilute with water to 50 ml
Allow to stand for five minutes	Allow to stand for five minutes
View downwards over a white surface	View downwards over a white surface

Observation:

The color produce in TEST(sample solution)should not be greater than STD (standard solution). If color produces in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa.

Method III: Use for the substance which gives **clear colorless** solution in sodium hydroxide solution.

Principle:

Limit test of heavy metals is based on the reaction of metallic impurities with sodium sulfide in alkaline medium to form brownish colour solution

Test sample	**Standard compound**
Solution is prepared as per the monograph and 25 ml is transferred in Nessler‟s cylinder or weigh specific amount of substance and dissolve in 20 ml of water and add 5 ml of dilute sodium hydroxide solution	Take 2 ml of standard lead solution
Make up the volume to 50 ml with water	Add 5 ml of dilute sodium hydroxide solution and make up the volume to 50 ml with water
Add 5 drops of sodium sulphide solution	Add 5 drops of sodium sulphide solution
Mix and set aside for 5 min	Mix and set aside for 5 min
View downwards over a white surface	View downwards over a white surface

Observation:

The color produce in sample solution should not be greater than standard solution. If color produces in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa.

Limit Test of Arsenic

Principle:

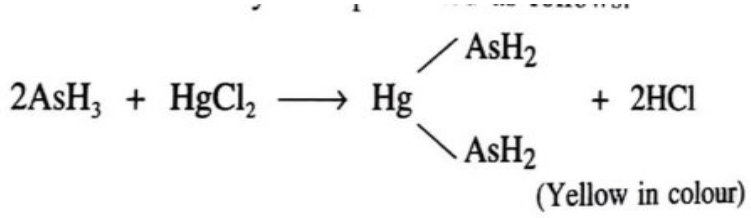
Limit test of Arsenic is based on the reaction of arsenic gas with hydrogen ion to form yellow stain on mercuric chloride paper in presence of reducing agents like potassium iodide. It is also called as **Gutzeit test** and requires special apparatus.

Arsenic, present as arsenic acid in the sample is reduced to arsenious acid by reducing agents like potassium iodide, stannous acid, zinc, hydrochloric acid, etc.

$$\underset{\text{Arsenic acid}}{H_3AsO_4} + H_2SnO_2 \rightarrow \underset{\text{Arsenious acid}}{H_3AsO_3} + H_2SnO_3$$

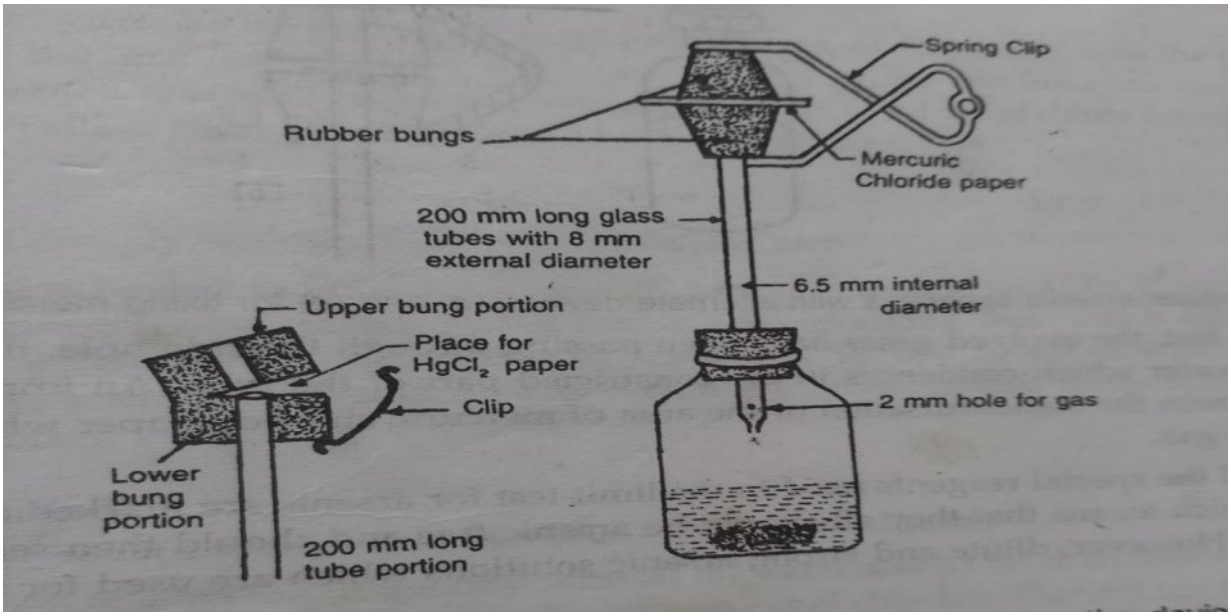
Arsenious acid is further reduced to arsine (gas) by hydrogen and reacts with mercuric chloride paper to give a yellow stain.

$$\underset{\text{Arsenious acid}}{H_3AsO_3} + 3H_2 \rightarrow \underset{\text{Arsine}}{AsH_3} + 3H_2O$$



APPARATUS:

- It consists of a wide mouthed glass bottle fitted with a rubber bung, through which passes a glass tube (length 200mm, outer diameter 8 mm, internal diameter 6.5 mm).
- This tube is open at its upper end but at the lower end it is drawn out to a smaller diameter (1 mm).
- A hole (2mm diameter) is blown in the side at its lower end, where it starts getting narrower.
- The purpose of this is to provide a small aperture at the lower end for the smooth and slow passage of arsine gas from the bottle to the tube.
- The purpose of the side hole is to provide an alternate passage for arsine if some water condenses at the narrower lower end.
- The glass tube is lightly packed with cotton wool which has been previously moistened with lead acetate solution and dried.
- The purpose of lead acetate cotton wool is to trap any hydrogen sulphide (H2S) gas which would otherwise interfere with this test as it also gives some stain with mercuric chloride paper.
- The tube is fitted at its upper end with two rubber bungs.
- A piece of dry mercuric chloride paper is placed flat on the top of the bung and other bung is placed over it and secured by means of clips in such a manner that the borings of the two bungs meet to form a true tube of the same diameter interrupted by a diaphragm of mercuric chloride paper.



Procedure:

Sl.no	TEST	STANDARD
1	Place 50 ml of distilled water in the bottle of an arsenic test apparatus labelled as TEST	Place 50 ml of distilled water in the bottle of an arsenic test apparatus labelled as STANDARD
2	Add 2.5 gm of sample (ammonium chloride) in the bottle and dissolve	Add 1 ml of dilute arsenic solution and mix.
3.	Add 10 ml of stannated hydrochloric acid	Add 10 ml of stannated hydrochloric acid
4.	Add 1gm of potassium iodide	Add 1 gm of potassium iodide
5.	Add 10 gm of grannulated zinc	Add 10 gm of grannulated zinc

Place the prepared glass tube quickly in position in both the cases and allow the action to proceed for 40 minutes in the dark.
Compare the yellow stain in the two, in day light without delay.

Observation

The intensity of yellow stain on mercuric chloride paper will depend upon the quality of arsenic present in the sample.

The intensity of the two stains is compared .

- If intensity of yellow stain produced in Test (sample solution) is less than the standard solution, the given sample passes the limit test of Arsenic and also the given sample complies with IP

- if the intensity of stain in the case of "TEST" is more than that of the "STANDARD" then the sample contains more arsenic than the limit hence sample is non-standard

Note

- All the reagents which are used for this test must be completely free from arsenic impurity.marked as AST

- Potassium iodide is used ,as it helps in reduction og pentavalent arsenic acid into trivalent arsenic acid
- If evolution of hydrogen gas is very slow, the two apparatus (test and standard) should be warmed at 40^0c by placing in water bath
- Grannulated zinc is used instead of ordinary zinc bcz evolution of nascent hydrogen is steady and prolonged with grannulated zinc

LIMIT TEST FOR LEAD

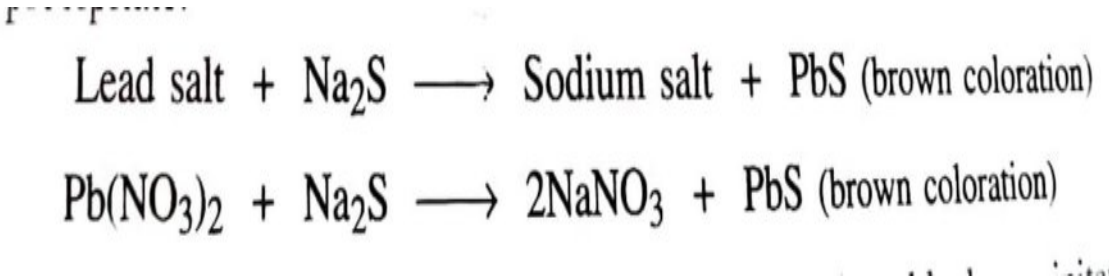
Lead is most undesirable in medicinal substances. Common source of lead in medicinal substances is due to its presence in sulphuric acid and in the apparatus which are used for their preparation. Defective storage can also increase the lead impurity in medicinal substances. If stored in ordinary lead glass bottles, likely to contain more quantity of lead as impurity especially when these are opened repeatedly in moist condition or kept without stoppers.

Two methods

- B P method
- IP/USP method

BP Method

Concentrated solutions of lead salts when treated with sodium sulphide give a black precipitate due to the formation of lead sulphide. Since very dilute solution of lead with sodium sulphide produce brown colouration and not black precipitate.



The intensity of the brown colour produced in the "Test" is compared with the "Standard". If it is more in Test , it means the sample contains more quantity of lead than permissible limit hence it is non-standard.

The test is carried out in Nesslers cylinder made up of lead free clear glass. All the reagents used should be completely free from lead and labeled as PbT

For this test the following two solutions are prepared:

1. Primary solution (test) : this contains a definite quantity of the test sample.
2. Auxillary solution (standard): this contains a very small quantity of test sample and specified volume of the standard lead solution

Sl no	Primary(test)	Auxiliary (standard)
1.	Place 12 g of ammonium chloride (sample) in Nesslers cylinder, labeled as " primary".	Place 2 g of ammonium chloride (sample) in Nesslers cylinder, labeled as " auxiliary". 2ml of dilute solution of lead is added
2.	Dissolve it in hot distilled water containing 5ml of acetic acid	Dissolve it in hot distilled water containing 5ml of acetic acid
3.	Make alkaline with solution of ammonia	Make alkaline with solution of ammonia
4.	Add 1 ml of potassium cyanide solution from burette	Add 1 ml of potassium cyanide solution from burette

Compare the intensity of colour in the two Nesslers cylinders. If there is any noticeable difference in colour it should be removed by adding very dilute solution of burnt sugar to the solution which has less intensity of colour.

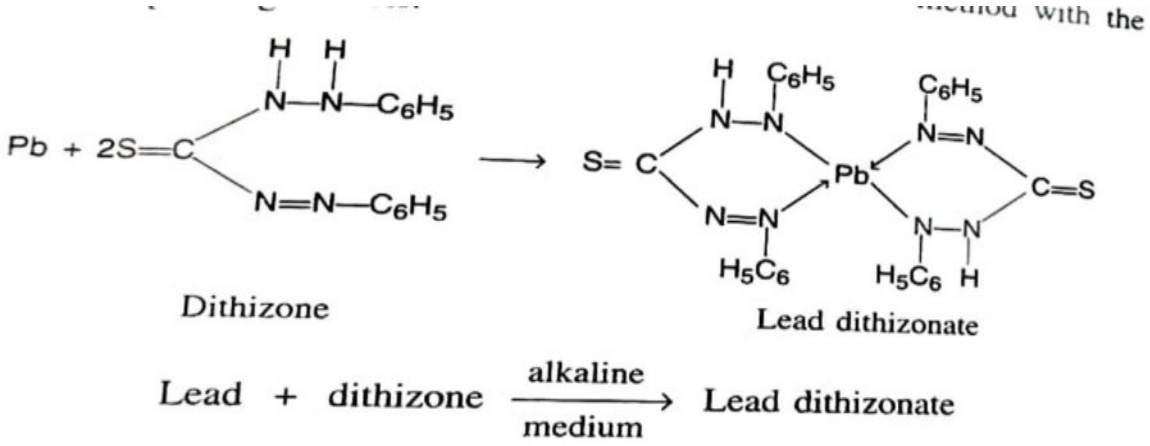
5.	Dilute the solution with distilled water to make 50 ml	Dilute the solution with distilled water to make 50 ml
6.	Add two drops of sodium sulphide solution	Add two drops of sodium sulphide solution

Stir each solution with a glass rod and allow to stand for 5 minutes. Compare the intensity of colour in the two Nesslers cylinders by viewing vertically downwards

IP/USP Method

Limit test for lead is based on the reaction between lead (impurity) and dithizone (diphenyl thiocarbazone) which results in the formation of lead –dithizonate.

This test is not carried out in Nesslers cylinders but involues double extraction method with the help of separating funnels.



$$\text{Lead} + \text{dithizone} \xrightarrow[\text{medium}]{\text{alkaline}} \text{Lead dithizonate}$$

Dithizone is soluble in chloroform and forms a green coloured solution. This is called dithizone-chloroform extract and is used for the quantitative extraction of lead from alkaline aqueous solution of the substance (test sample).

Final product (lead –dithizonate) in chloroform is red in colour. Mixture of these two(green + red) appears as violet in colour.

Observation

Intensity of the final violet colour produced in the chloroform medium in the case of the test sample should not be more than that in the standard.

References

Pharmaceutical inorganic chemistry- Chatwal
Pharmaceutical inorganic chemistry-Chaudary and kurban
Pharmaceutical inorganic chemistry-Rajesekharan